The Molecular Biology of Chromosome Alterations in Myelogenous Leukemia

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In the past few years, new developments have rapidly emerged that promise to unravel the pathogenesis of malignant transformation. By understanding the molecular events in neoplastic transformation, such as in myelogenous leukemia, we should be able to devise specific and effective therapies and to intervene in the development of these diseases. Furthermore, the study of cancer genes is likely to have far-reaching effects with regard to our understanding of normal development and other nonneoplastic conditions.

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A large body of literature has rapidly emerged concerning the isolation and characterization of transforming genes, or oncogenes, that appear to be at least in part responsible for the development of various malignant diseases. These advances have been made possible by basic research in a wide range of disciplines, many of which had no initial obvious connection with cancer per se. DNA transfection experiments, which consist of introducing tumor DNA into recipient cells and isolating transformed foci, have shown the frequent occurrence of an altered *ras* oncogene product in a wide variety of tumors, including acute leukemia. Thus, the *ras* oncogene appears to be commonly involved in either tumor initiation or tumor progression. The observation that chromosome rearrangements were nonrandomly associated with specific malignant diseases suggested that genes located at or near the sites of these rearrangements would be involved in the transformation process. Before discussing some of the developments in the myeloid leukemias, it is worth reviewing one of the most studied malignant diseases to date, namely Burkitt's lymphoma.

Burkitt's lymphoma is characteristically associated with a reciprocal exchange of chromosome material between chromosomes 8 and 14. Less frequently, this translocation occurs between chromosomes 2 and 8, or chromosomes 8 and 22. One of these translocations is present in virtually every case of Burkitt's lymphoma. Through the technique of somatic cell genetics, it was possible to separately isolate these chromosomes in rodent-human cell hybrids.[1] What was found was that the c-*myc* oncogene normally located on chromosome 8 at the site of rearrangement had been translocated next to immunoglobulin genes on chromosome 14. In the case of the variant 8;22 and 2;8 translocations, the c-*myc* oncogene remained on chromosome 8 but was juxtaposed to immunoglobulin genes from either chromosome 2 or 22, which had moved to the other side of c-*myc*.[2,3] Using recombinant DNA techniques, several investigators were able to clone and identify the actual site of recombination from different Burkitt's lymphomas and to determine the effect of the translocation on the expression (messenger RNA) of c-*myc*. Although significant variations in the actual site of recombination were observed, a unifying concept emerged that deregulation of c-*myc* expression resulted from the translocation.[4,5] The product of the c-*myc* gene is a nuclear binding protein that is intimately associated with cell proliferation.[6] It is felt that increased cell division is a result of deregulated c-*myc* expression. In Burkitt's lymphoma, the altered c-*myc* expression, due to the influence of immunoglobulin genes, is tissue specific and, depending on the translocation, may be differentiation specific as well,[4,7] Thus, the translocated c-*myc* gene is only abnormally expressed in B lymphocytes. This is an important observation and has broad implications for the isolation of transforming genes using a transfection assay. The existence of cloned immunoglobulin gene probes allowed the isolation of a new flanking DNA sequence that in this case turned out to be c-*myc*.

Recently, two additional translocations have been isolated and cloned in a similar manner. The 14;18 translocation is present in most cases of nodular lymphomas and the 11;14 translocation is seen in diffuse small and large cell lymphomas.[8,9] In both cases, putative oncogenes from chromosomes

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11 and 18 have been isolated.[10,11] Like c-*myc*, these genes have been translocated to the immunoglobulin heavy-chain region on chromosome 14 that facilitated their cloning. The normal function of these genes and functional changes resulting from the translocation are under intense investigation.

With this background in mind, we shall now examine some of the molecular alterations in the myeloid leukemias.

Chronic Myelogenous Leukemia and the Philadelphia (Ph¹) Translocation

Chronic myelogenous leukemia is associated with a specific translocation, t(9;22)(q34;q11), in more than 90% of patients.[12] In the remaining cases, either a three-way variant translocation occurs, or there is no translocation—that is, Ph¹-negative chronic myelogenous leukemia. The Ph¹ chromosome refers to a deletion in the long arm (q) of chromosome 22 resulting from the translocation of 22q material to the long arm of chromosome 9. This was initially shown by Rowley and was the first specific chromosome abnormality identified in malignant disease.[13] In patients with chronic myelogenous leukemia, a chronic phase usually persists for about three years, followed by an accelerated phase ending in blast crisis. This accelerated and blast crisis phase is associated with additional nonrandom chromosome alterations. The most frequent changes are a second Ph¹ chromosome; an isochromosome 17, consisting of both long arms of 17 joined at the centromere, and an extra chromosome 8. These secondary changes appear to represent a mechanism of tumor progression.

Using somatic cell hybrids and in situ hybridization experiments, investigators were able to determine that the c-*abl* oncogene, normally located on the long arm of chromosome 9, had been translocated to the 22q− chromosome.[14] In fact, in variant three-way translocations, this is the constant feature, namely, the movement of c-*abl* to the 22q−.[15] In Ph¹-negative chronic myelogenous leukemia, there is no translocation of c-*abl*.[14] This is consistent with the concept that Ph¹-negative chronic myelogenous leukemia is a different disease associated with a poorer prognosis.

Investigators were able to clone the actual site of rearrangement, which led to an important observation. The normal c-*abl* oncogene is large (32 kilobases [kb]) and the breakpoint occurs in the 5′ portion of the gene, although the site of rearrangement varies considerably among different cases of chronic myelogenous leukemia. The chromosome 22 region where the break occurs is quite limited, however.[16] Most rearrangements can be shown to occur within a 5.8-kb segment, referred to as the breakpoint cluster region, or bcr. This finding suggests that both chromosome regions are critical to the biology of the translocation. Unlike the Burkitt's lymphoma translocation, the chromosome 22 bcr region has no homology to immunoglobulin genes. Neither is there homology to the c-*sis* oncogene that has been mapped to chromosome 22, although more distal to the breakpoint. In fact, there is no evidence to date that c-*sis* has any role in the development of chronic myelogenous leukemia.

Despite the variability in the c-*abl* breakpoint, the translocation results in a single new species of c-*abl* RNA.[17] Nonchronic myelogenous leukemia hematopoietic cells contain two c-*abl* RNA species of 6 and 7 kb. In chronic myelogenous leukemia, a new 8-kb message can be detected, either as the only RNA species or in combination with the 6- and 7-kb transcripts. This larger message results from a chimeric or fused gene consisting of the bcr region joined to the altered c-*abl*. The obvious question is how this alteration might be pathogenic. The c-*abl* oncogene also exists, in an altered form, in an acutely transforming RNA tumor virus. This virus produces predominantly an acute pre-B-cell leukemia. The transforming ability of this v-*abl* gene is due to a tyrosine kinase activity in the v-*abl* protein. Using similar assays, however, the normal c-*abl* gene does not possess tyrosine kinase activity. The change in function between the normal cellular gene and the viral oncogene appears to result from an alteration in the 5′ portion of the gene. In chronic myelogenous leukemia, the abnormal 8-kb messenger RNA gives rise to a larger than normal 210,000-dalton protein referred to as P210 c-*abl*. Like the viral *abl* protein, P210 c-*abl* also has tyrosine kinase activity. It is believed that an alteration in the 5′ region of the gene, resulting from the translocation, unmasks or deregulates this kinase activity, leading in part to the development of chronic myelogenous leukemia.[18] This abnormal protein may provide a specific target for future therapy. Compared with the Burkitt's lymphoma translocation that results in abnormal expression of a normal protein, the chronic myelogenous leukemia translocation results in a qualitative alteration in the protein. Again, despite heterogeneity in the actual site of recombination, a common alteration appears to result. This is important and suggests that specific interventions may be effective in most patients who have chronic myelogenous leukemia.

The development of malignancy is felt to be a multistep process. Chronic myelogenous leukemia appears to be a case in point where there is some evidence that alterations may occur before the translocation arises. In a multistep pathway, it may not be necessary to uncover every step before discovering a solution. Of course, the more information that is known regarding these alterations, the higher is the likelihood of developing such an intervention.

The M2 Subtype of Acute Myeloblastic Leukemia and the 8;21 Translocation

Before the advent of chromosomal banding, a group of patients with acute myeloblastic leukemia was described possessing a chromosome abnormality referred to as the complex profile and similar disease characteristics. With quinacrine banding, Rowley was able to show that this abnormality represented a reciprocal translocation between chromosomes 8 and 21 at the bands 8q22.1 and 21q22.3.[15] Of interest, this translocation is specific for the M2 subtype of acute myeloblastic leukemia—that is, acute myeloblastic leukemia with maturation. Of patients with de novo acute myeloblastic leukemia and the M2 subtype, approximately 18% have the t(8;21). If one considers only those patients with the M2 subtype and detectable chromosome abnormalities, then about a third have the t(8;21). Of particular interest is that the chromosome 21 region involved in this translocation is the region required to be trisomic to produce the Down syndrome phenotype. Moreover, patients with Down's syndrome have a greatly increased risk of leukemia. Another observation is that neonates with Down's syndrome may have at birth what appears to be acute leukemia, except that spontaneous resolution of this syndrome is the rule. Some of these persons have

shown a mosaic pattern for trisomy 21. In these cases, the "blast cells" contained the trisomy 21 clone, suggesting that this population of cells possessed a growth advantage. Furthermore, in complex three-way 8;21 translocations, the constant feature is the movement of chromosome 21 material to the 8q− chromosome.[15]

These observations suggest that a gene(s) on chromosome 21 may be involved in the growth and regulation of hematopoietic cells. We have isolated both translocation chromosomes in separate somatic cell hybrids. We have found that there is an oncogene on chromosome 21, that it is in the appropriate region and that it is translocated to the 8q− chromosome in this disease. (The details of this will be published elsewhere.)

An additional oncogene, c-*mos*, has been localized to the region of chromosome 8 where the rearrangement occurs.[19] For this gene, we have shown that it remains on chromosome 8, and we have not detected any rearrangements in several patients examined so far.[20] Because a cytogenetic band contains about 5 million base pairs of DNA, it is easy to see how translocations may involve a particular band and yet be located at a great distance from a similarly localized DNA sequence.

Until recently, c-*mos* RNA had never been detected in any human tissue, tumor or cell line. Recently, investigators have been able to detect c-*mos* transcripts in a variety of tissues.[21] The highest level seems to be present in gonadal tissue, and different-sized transcripts are present in different tissues.

The 8;21 translocation of acute myeloblastic leukemia is associated with a high incidence of the loss of a sex chromosome. This loss has been shown to be a secondary change developing after the 8;21 translocation.[22] The X and Y chromosomes are known to contain some gene duplications. Although speculation, the function of c-*mos* may have to do with some aspect of sexual function or development. The loss of a sex chromosome might conceivably influence c-*mos* expression. Alternatively, altered c-*mos* expression may result from the 8;21 translocation and could be further influenced by such a loss. Since c-*mos* expression has now been reported, it should be possible to answer such questions. Present information suggests that c-*mos* has protein kinase activity. Unlike c-*abl* and c-*src* kinase activities that phosphorylate predominantly tyrosine residues, c-*mos* phosphorylates serine and threonine residues. Recent evidence has shown that c-*mos* has homology to the epidermal growth factor (EGF) precursor molecule and also to a cell-cycle-specific gene in yeast, CDC-28.[23,24] It is postulated that the pro-EGF molecule might function in cell-cell interactions.

It should be noted that the chromosome 21 oncogene may be involved in producing the Down syndrome phenotype. Again, the study of cancer genes without doubt will greatly influence our understanding of many other abnormalities and vice versa.

Acute Promyelocytic Leukemia and the 15;17 Translocation

Acute promyelocytic leukemia has been of particular clinical importance because of its high association with disseminated intravascular coagulation. Recognition of this subtype is important because low-dose heparin therapy may prevent fatal hemorrhage. A microgranular variant has been described wherein the granules are too small (<250 nm) to be seen by light microscopy. Acute promyelocytic leukemia is characteristically associated with a reciprocal translocation between chromosomes 15 and 17, t(15;17)(q22;q21.1). There is good evidence that all patients with acute promyelocytic leukemia have the t(15;17). The *erb*-A oncogene has been localized on chromosome 17 proximal to the breakpoint, but there is yet no evidence linking *erb*-A to acute promyelocytic leukemia.[25] Chromosome 15 also contains the c-*fes* oncogene, but this is located far distal to the breakpoint. Recently, the cellular tumor antigen p53 has been localized to bands 17q21-q22, the region where the breakpoint occurs, and is translocated to the 15q+ chromosome. A role for p53 in acute promyelocytic leukemia is suggested by this information, and, moreover, this appears to be the conserved abnormality in variant translocation.[26]

Acute Myelomonocytic Leukemia (M4) With Associated Bone Marrow Eosinophils and Abnormalities of Chromosome 16

LeBeau and others have described a rearrangement of chromosome 16, frequently a pericentric inversion, inv(16)(p13q22), that is highly correlated with a variant type of myelomonocytic leukemia.[27] In this variant, there are cells with eosinophilic characteristics but with abnormal morphology and cytochemistry. The nucleus may be folded, resembling a monocyte, and the cytoplasmic granules may be predominantly eosinophilic or basophilic. The positive reaction of these granules with periodic acid-Schiff and chloracetate esterase is atypical. It may be that these cells represent stages of differentiation in the leukemia cell. Of clinical interest is that patients with this pattern appear to have a prolonged remission.

The metallothioneins are proteins known to bind and protect against heavy metals. These proteins may also function as regulators of growth and differentiation by binding enzyme requiring metal ions.[28] The chromosome 16 inversion has been shown to split the cluster of metallothionein genes present on chromosome 16. Some of these patients have a rearrangement in the gene structure demonstrable by Southern blots. Investigators are attempting to isolate one of the rearranged genes and to determine if an oncogene has been activated.

Abnormalities of 11q in Monocytic Leukemia

Translocations or deletions in the long arm of chromosome 11 appear to be associated predominantly with monocytic leukemia.[12] The breakpoint is usually in band 11q23-24, but may also be in 11q13-14. The most common translocation involves the short arm of chromosome 9 at p22 and the long arm of chromosome 11 at q23—that is, t(9;11)(p22;q23). Of interest, the *ets* oncogene is normally located on chromosome 11 at band q23. In situ hybridization studies have recently shown that this oncogene is translocated to chromosome 9, although a rearrangement of the gene was not detected in this particular case.[29] The oncogene *ets* was isolated from the RNA tumor virus E26. This virus carries two oncogenes, *myb* and *ets*. Another retrovirus, avian myeloblastosis virus, produces a pure myeloid leukemia and contains only the *myb* oncogene. The E26 virus produces predominantly an erythroid leukemia, although transformed myeloid cells can be

shown. The *ets* gene is apparently responsible for the enhanced spectrum of activity.[30]

A high correlation has recently been described between acute lymphoblastic leukemia with bulky disease and abnormalities of the short arm of chromosome 9.[31] This region is the same one involved in the 9;11 translocation described above. Thus, isolation of the 9;11 breakpoint may have implications for acute lymphoblastic leukemia as well.

Secondary Leukemias and Myelodysplastic Syndromes

The secondary leukemias and myelodysplastic syndromes constitute a heterogenous group of diseases and will not be covered in detail. The development of secondary leukemia or a myelodysplastic syndrome after treatment is usually fatal and is an all-too-frequent complication. The large majority of such cases have a deletion of part or all of chromosome 5, chromosome 7 or both. These chromosome abnormalities also occur in de novo acute myeloblastic leukemia but usually in an older age patient. This suggests that a similar cause may be involved in both groups.

A myelodysplastic syndrome referred to as the "5q-syndrome" consists of refractory anemia, myeloid hyperplasia, hyperlobulated megakaryocytes and peripheral thrombocytosis. Many of these patients have progression to acute leukemia. The oncogene c-*fms* has localized to chromosome 5 near the deletion. A 5q− chromosome has been isolated from one patient and loss of the c-*fms* oncogene was shown. The c-*fms* oncogene is related to and may be identical with the receptor for the hematopoietic growth factor CSF-1.[32] This factor is involved in the differentiation and proliferation of primitive hematopoietic cells. It is possible that an abnormality of the CSF-1 receptor could lead to myelodysplasia. This is an exciting observation that is likely to shed light on this group of diseases.

In summary, significant and rapid progress in understanding the pathogenesis of malignant disease has been made, but we are witnessing only the beginnings of the results. It is to be stressed again that cancer research and other basic research go hand in hand. The goal of this research is to understand the basic mechanisms that will lead to specific therapies and interventions to interrupt the disease process. Such research will have broad implications for many diseases and developmental abnormalities as well.

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